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Effect of Benzo(a)pyrene on Sister-Chromatid Exchange in Fetal Hamster Liver Exposed in Utero

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Effect of benzo[*a*]pyrene on sister-chromatid exchange in fetal hamster liver exposed in utero*

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Summary

Benzo[*a*]pyrene between 50 and 125 mg/kg administered maternally caused a dose-related increase in sister-chromatid exchange in fetal hamster liver cells. There was no difference on days 11, 13 and 15 of gestation in the sensitivity of fetal liver to benzo[*a*]pyrene.

The in utero exposure of the fetus to chemical mutagens can result in fetotoxicity, neoplasia, congenital anomalies and heritable abnormalities. The induction of sister-chromatid exchange (SCE) has been demonstrated to be a sensitive procedure for the detection of mutagens and agents capable of causing chromosomal damage (Latt et al., 1981). SCE represents the exchange between 2 sister chromatids at homologous loci of genetic material. Although the molecular mechanism of SCE is unknown, it is presumed that the induction of SCE resulted from the damage and alteration of DNA. The in vivo SCE has been extended to include the detection of chemical induced SCE in rodent fetus exposed in utero (Basler, 1979; Kram et al., 1979, 1980; Knuutila et al., 1979; Allen et al., 1981).

Benzo[*a*]pyrene has been demonstrated to enhance the incidence of SCE in the liver of partially hepatectomized mice (Schreck and Latt, 1980) and in cultured mouse embryos (Galloway et al., 1980). In this communication, we examined the induction of SCE in Syrian hamster fetal liver by in utero exposure to benzo[*a*]pyrene at different ages of gestation.

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Materials and methods

Chemicals

Cyclophosphamide, 5-bromo-2′-deoxyuridine (BrdU) and trioctanoin were purchased from Sigma Chemical Co., St. Louis, MO; benzo[*a*]pyrene (BaP; Gold label; 99+% purity) from Aldrich Chemical Co., Milwaukee, WI; dimethyl sulfoxide (DMSO) from Burdick and Jackson Lab., Inc., Muskegon, MI, and Hoechst 33258 from Calbiochem.-Behring Corp., La Jolla, CA.

Animals

Golden Syrian hamsters weighing 80–100 g were purchased from Engle's Laboratory Animals, Farmersburg, IN. The animals were maintained in accordance with the standards set forth in the 'Guide for the Care and Use of Laboratory Animals' of the Institute of Laboratory Animal Resources, National Research Council. They received Purine Laboratory Chow (Ralston Purina Co.) and water ad libitum.

SCE in fetal liver

Pregnant Syrian hamsters were treated with BrdU by the pellet implantation method of Allen et al. (1978). Pellets weighing 300 mg were prepared with a Parr press (Parr Instrument Co.). While the animals were under light ether anesthesia, the pellet was surgically implanted under the skin in the interscapular area. 2 h later, BaP was administered by intraperitoneal injection. 18 h after treatment were given, the pregnant animals were sacrificed by cervical dislocation.

The fetuses were quickly removed from the uteri and the livers from each litter (10–14 fetuses) pooled in 10 ml of Ca^{2+} and Mg^{2+} free Hank's Basal Saline Solution (HBSS). After incubation for 20 min at 37°, the livers were gently crushed with a spatula against the side of the test tube in order to release the cells from the connective tissue. The cell suspension was filtered through 2 layers of nylon mesh, 250 and 62 μm in porosity. Next, colcemid (0.1 μm/ml) was added and the suspension incubated at 37°C for 2 h in a CO_2 incubator. The cells were collected by centrifugation at 500 rpm for 10 min. The pellet was resuspended into 5–6 ml of 0.075 M KCl by thumping the tube and incubated for 5 min. The cells were collected by centrifugation and resuspended in ice-cold Carnoy's fixative (methanol–acetic acid, 3:1) and held at 4° for 20 min. After 2 successive changes of the fixative, the cells were dropped on ice-cold, and wet slides and air-dried overnight.

The cells were stained by the Hoechst–black light–Giemsa method described by Goto et al. (1978). The slides were stained in Hoechst 33258 (0.05 mg/ml in distilled water) for 15 min, blotted and covered with MacIlvaine's buffer. While on a 50°C slide warmer; the slides were subjected for 22 min to black-light irradiation. They were then rinsed, dried at 37°C for at least 1 h and stained in 2% Giemsa (Gurr's R66, Biomedical Specialties) for 10 min. Sister-chromatid exchanges were counted

in 25 metaphase spreads per litter using 1000 × magnification. Mitotic index was determined by counting the number of dividing cells per 500 cells.

Results

The effect of BaP administered intraperitonally to pregnant hamsters on the incidence of SCE in the liver of their fetuses is presented in Table 1. The baseline level

TABLE 1

EFFECT OF BENZO[*a*]PYRENE ON SISTER-CHROMATID EXCHANGE IN THE HAMSTER FETAL LIVER CELLS

Treatment dose (mg/kg)	ACE/metaphase Age of fetus (days)		
	11	13	15
1. Benzo[*a*]pyrene			
a. 0[a]	6.2 ± 0.28[b]	6.5 ± 0.34	6.4 ± 0.39
b. 50	9.3 ± 0.50	9.5 ± 0.50	9.4 ± 0.53
c. 100	11.5 ± 0.71	11.3 ± 0.45	11.7 ± 0.60
d. 125	13.4 ± 0.62	12.5 ± 0.65	13.9 ± 0.83
2. Cyclophosphamide			
a. 10	12.2 ± 0.54	13.7 ± 0.66	14.6 ± 1.01

[a] The hamsters received the solvent (Trioctanoin) for the benzo[*a*]pyrene and cyclophosphamide.
[b] The fetal livers from each litter were pooled and the results are the means ± SE for 5 litters. 25 metaphases were scored for each litter.

TABLE 2

EFFECT OF BENZO[*a*]PYRENE ON MITOTIC INDEX IN THE HAMSTER FETAL LIVER CELLS

Treatment (mg/kg)	Dividing cells/500 cells Age of fetus (days)		
	11	13	15
1. Benzo[*a*]pyrene			
a. 0[a]	36.0 ± 0.9[b]	45.6 ± 0.7	22.2 ± 0.9
b. 50	37.0 ± 0.5	45.6 ± 1.2	24.0 ± 0.6
c. 100	33.4 ± 0.8	40.0 ± 0.3	21.2 ± 0.4
d. 125	23.8 ± 0.6	28.8 ± 1.0	15.6 ± 0.7
2. Cyclophosphamide			
a. 10	27.6 ± 0.9	32.8 ± 0.6	17.8 ± 0.9

[a] The hamsters received the solvent (Trioctanoin) for the benzo[*a*]pyrene and cylophosphamide.
[b] The fetal livers from each litter were pooled and the results are the means ± SE for 5 litters. A total of 500 cells were scored for each litter.

of SCE was consistent at 11, 13 or 15th day of gestation. BaP, at 50 mg/kg or higher, significantly increased the incidence of SCE in the fetal liver cells (2-tail Student t test with $P<0.05$). A dose of 125 mg/kg BaP resulted in a doubling of the incidence of SCE compared to the incidence in fetal liver from untreated hamsters. Cyclophosphamide (10 mg/kg) at all 3 ages of gestation also induced a doubling in the incidence of SCE compared to untreated controls (Table 1). There was no difference in the sensitivity of 11, 13 and 15 day old fetuses to the induction of SCE by either BaP or cyclophosphamide.

BaP caused a dose-related reduction in the mitotic index in fetal hamster liver on days 11, 13 and 15 of gestation (Table 2). Cyclophosphamide (10 mg/kg) also reduced the mitotic index at all 3 ages of gestation. The mitotic activity in the fetal liver from untreated hamsters was less on day 15 of gestation than either days 11 or 13.

Discussion

Fetal exposure to genotoxic chemicals can result in fetotoxicity, carcinogenesis, teratogenesis and heritable abnormalities. The induction of SCE in transplacentally exposed fetuses has been demonstrated and proposed as an assay to screen for fetal response to genotoxic chemicals (Allen et al., 1981; Latt et al., 1981). BaP induced a dose-related increase in the incidence of SCE in fetal hamster liver that resulted in a doubling of the incidence at a dose of 125 mg/kg BaP. Another short-term assay proposed to screen chemicals for genotoxic activity was the micronucleus assay, which was insensitive to a higher dose of 500 mg/kg BaP (Heddle and Bruce, 1977). The SCE assay was also demonstrated to be a more sensitive indicator of exposure to genotoxic chemicals than was chromosomal aberrations (Perry and Evans, 1975; Wolff, 1977).

Under our experimental conditions, there was no significant difference in the sensitivity to either BaP or cyclophosphamide in fetal hamster liver on days 11, 13 or 15 of gestation. Kram et al. (1980) reported in mice that the sensitivity of fetal cells to cyclophosphamide and mitomycin C starting from day 11 of gestation decreased with gestational age. This difference may represent species variation in the sensitivity to cyclophosphamide. Kram et al. (1980) also observed that fetal liver was relatively more sensitive than the lung and gut cells to cyclophosphamide, a drug which requires metabolic activation. There was, however, no difference in the sensitivity of mouse fetal liver, lung and gut cells in terms of SCE induced by direct-acting genotoxic agents. These observations suggest that fetal liver cells may be more sensitive to genotoxic agents requiring metabolic activation. Furthermore, in mice, fetal liver (Kram et al., 1980) and partially hepatectomized liver (Roberts and Allen, 1980) were more sensitive to cyclophosphamide and ethyl carbamate, respectively, than the bone-marrow cells. Therefore, the rapidly dividing liver cells present in

fetal and regenerating liver would appear to be the organ of choice to screen chemicals that require metabolic activation in order to induce SCE.

In conclusion, the analysis of SCE in fetal liver cells seems to be a sensitive short-term assay to screen chemicals for the detection of genotoxic agents that are active transplacentally.

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